IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket. No. 041082-0113

Applicant:	Aser ROTHSTEIN *et al.*
Title:	SELF-ALIGNING PEPTIDES MODELED ON HUMAN ELASTIN AND OTHER FIBROUS PROTEINS
Application No.:	Divisional of Serial No. 09/340,736
Filing Date:	Filed Concurrently Herewith
Examiner:	Unassigned
Art Unit:	Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-captioned application, Applicants respectfully request that the application be amended as follows:

IN THE SPECIFICATION

1. At page 1, after the Title, please insert the following information:

CROSS REFERENCE TO RELATED APPLICATION

This application is a divisional of U.S. patent application serial No. 09/340,736, filed June 29, 1999, which is a continuation-in-part of U.S. patent application serial No. 08/911,364, filed August 7, 1997, now U.S. patent No. 5,969,106, which, in turn, is based on U.S. provisional patent application serial No. 60/023,522, filed August 7, 1996. The entire contents of these applications, including their specifications, claims and drawings, are incorporated herein by reference in their entirety.

2. At page 2, line 36 – page 3, line 10, please delete the entire paragraph and replace with the following:

U.S. Patent No. 4,474,851 (Urry) is directed to an elastomeric composite material comprising an artificial core fiber, such as Dacron, and a polypeptide comprising repeating

tetrapeptide or pentapeptide units. The units are derived from units observed to be repeated in the tropoelastin molecule, Val-Pro-Gly-Val-Gly (VPGVG; SEQ ID NO:6) and Val-Pro-Gly-Gly (VPGG; SEQ ID NO:7). The polypeptide comprises a series of beta-turns and is proposed to have a beta-coil structure. The polypeptide provides elastomeric properties to the composite material, but has little structural strength or integrity. The artificial core fiber provides these latter properties to the composite material.

3. At page 3, second full paragraph, please delete the entire paragraph and replace with the following:

Elastin-based materials also have been used to produce solid materials from which prostheses can be manufactured. These include soluble animal elastin co-aggregated with other proteins such as collagen, fibrin, fibronectin and laminin, to produce gel-like materials, and polymerized materials derived from short hydrophobic sequences of human elastin (such as PGVGVA; SEQ ID NO:5). In some cases, these synthetic peptides also include short alanine-rich sequences containing lysine residues, allowing cross-linking between the elastin-like peptides or to other proteins such as collagen. Both elastin and collagen contain crosslinks derived from lysine. For example, U.S. Patent No. 5,223,420 (Rabaud) is directed to an elastin-based product comprising an adduct containing elastin and at least one other protein, such as fibrin.

4. At page 3, line 32 – page 4, line 7, please delete the entire paragraph and replace with the following:

U.S. Patent No. 4,589,882 (Urry) is directed to an artificial elastomeric copolymer comprising an elastomeric component of repeating units of tetrapeptides and pentapeptides and a crosslinking component which may comprise amino acid residues. The repeating units are derived from elastin. U.S. Patent No. 4,132,746 (Urry) is directed to a synthetic, insoluble, crosslinked polypentapeptide. The pentapeptide is the VPGVG (SEQ ID NO:6) peptide present in tropoelastin. See also U.S. Patent No. 4,500,700, U.S. Patent No. 4,870,055, and U.S. Patent No. 5,250,516 (all to Urry) for other materials derived from this peptide. The polypeptides described in these patents comprise a series of beta-turns and are proposed to have a beta-coil structure.

5. At page 6, lines 34-37, please delete the entire paragraph and replace with the following:

Figure 1B shows the amino acid sequence of human elastin (SEQ ID NO:1), without the signal peptide. The underlined amino acid residues comprise the polypeptide of the present invention named MFU-1.

6. At page 7, lines 15-17, please delete the entire paragraph and replace with the following:

Figure 4C shows the amino acid sequence of MFU-2 (SEQ ID NO:2).

7. At page 7, line 21, please insert the following paragraph:

Figures 5A, 5B and 5C show the amino acid sequences of MFU-3 (SEQ ID NO:9), MFU-4 (SEQ ID NO:10), and MFU-5 (SEQ ID NO:11), respectively.

8. At page 8, line 32 – page 9, line 5, please delete the entire paragraph and replace with the following:

Tropoelastin consists predominantly of alternating hydrophobic and crosslinking domains. Indik *et al.*, *Proc. Nat'l Acad. Sci. USA* 84: 5680-84 (1986). Crosslinking domains are rich in alanine (A), with the lysines (K) destined for involvement in crosslink formation present in KAAK (SEQ ID NO:3) and KAAAK (SEQ ID NO:4) spacings. The domains separating these crosslinking regions are strongly hydrophobic in character, and contain many tandemly repeated penta- and hexa-peptide sequences. In human elastin the most striking of these is the sequence PGVGVA (SEQ ID NO:6), repeated 7 times in exon 24. Indik *et al.*, *supra*.

9. At page 9, line 31 – page 10, line 17, please delete the entire paragraph and replace with the following:

As shown in Figure 1A, human elastin consists for most of its length of alternating crosslinking domains and hydrophobic domains. The crosslinking domains consist mainly of lysine (K) and alanine (A) residues in KAAK (SEQ ID NO:3) and KAAAK (SEQ ID NO:4) sequences, wherein the lysine residues are in a suitable conformation for oxidative deamination by lysyl oxidase and subsequent formation of the covalent desmosine crosslinks.

Indik *et al.*, *supra.* The hydrophobic domains are rich in hydrophobic pentapeptide and hexapeptide sequences believed to be in beta-sheet/beta-turn structures. Tamburro *et al.*, ADVANCES IN LIFE SCIENCES 115-27 (1990). These hydrophobic regions are believed to be important to elastin's physical properties of extensibility and elastic recoil, and to the ability of tropoelastin (the monomeric precursor of elastin) to self-aggregate into fibrillar structures. Robson *et al.*, *supra*; Tamburro *et al.*, *supra.* Other proteins capable of self-aggregation and self-alignment into stable fibrillar matrices, including eggshell chorion proteins of insects, spider dragline silk, and lamprin from lamprey cartilage, all possess similar regions of hydrophobic repeat peptides with beta-sheet/beta-turn structures. Hamodrakas *et al.*, *Int. J. Biol. Macromol.* 11: 307-13 (1989); Simmons *et al.*, *Science* 271: 84-87 (1996); Robson *et al.*, *supra.*

10. At page 13, line 23 to page 14, line 16, please delete the entire paragraph and replace with the following:

In accordance with one embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1). The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1), wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10 amino acid residues, for example, from 1 to about 5 amino acid residues. Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. In accordance with another embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of the amino acid sequence set forth in Figure 4C (SEQ ID NO:2). The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid sequence set forth in Figure 4C (SEQ ID NO:2), wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10 amino acid residues, for example, from 1 to about 5 amino acid residues. Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. Polypeptides whose amino acid sequences are variants of the amino acid sequences set forth in Figures 5A-5C (SEQ ID NOS:9-11, respectively) also are encompassed by the present invention. The amino acid sequences of such polypeptides comprise a portion of an amino acid sequence set forth in Figure 5A, 5B or 5C (SEQ ID NOS:9, 10 or 11, respectively),

wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues, for example, from 1 to about 5 amino acid residues. Such polypeptides have a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibit the properties of self-alignment discussed herein.

11. At page 14, second full paragraph, please delete the entire paragraph and replace with the following:

The domain structure of human elastin is illustrated in Figure 1A. As shown in this Figure, there are a number of alternating crosslinking and hydrophobic domains. The hydrophobic domains each are believed to comprise a number of beta-sheet/beta-turn-forming sequences. These domains represent probable MFUs of elastin. One of these, used in further experimentation, is designated by the bracket and is named MFU-1 (see Example 1 below). Figure 1B sets forth the amino acid (SEQ ID NO:1) of human elastin. The underlined amino acid residues, residues 374-499, comprise MFU-1. Other MFUs modeled on human elastin include polypeptides comprising amino acid residues 19-160, 188-367 and 607-717, respectively. The amino acid sequence of MFU-3 (SEQ ID NO:9; Fig. 5A) corresponds to that of MFU-1 without the first five amino acid residues. The amino acid sequence of MFU-4 (SEQ ID NO:10; Fig. 5B) corresponds to that of MFU-1 without the first four amino acid residues.

12. At page 14, line 33 – page 15, line 5, please delete the entire paragraph, and replace with the following:

MFUs modeled on human elastin comprise a portion of the amino acid sequence of the tropoelastin molecule (Figure 1B; SEQ ID NO:1) and have at least three beta-sheet/beta-turn structures in their secondary structure. They also may comprise amino acids residues which are capable of participating in crosslinking, such as lysine residues. In one embodiment of the invention, the MFU comprises two amino acid residues capable of participating in crosslinking in such a manner as to form a desmosine-type linkage. For example, the MFU may comprise a KAAK (SEQ ID NO:3) or KAAAK (SEQ ID NO:4) amino acid sequence.

13. At page 15, first full paragraph, please delete the entire paragraph and replace with the following:

In a preferred embodiment, a polypeptide modeled on human elastin consists essentially of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1). The phrase "A consists essentially of B" herein denotes that A comprises B and possibly other components that do not materially affect the characteristics of the A-B material. For example, a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) denotes a polypeptide which comprises a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) and which also may comprise other amino acid residues that do not materially alter the characteristics of the polypeptide. That is, the polypeptide maintains the characteristics of having at least three beta-sheet/beta-turn structures, and self-aligning in the same manner as tropoelastin peptides.

14. At page 16, first full paragraph, please delete the entire paragraph and replace with the following:

By means of available technology, DNA sequences coding for tandem repeats of any human elastin MFU, or for MFUs containing larger domains of human elastin, up to and including the entire tropoelastin molecule, can be constructed. These larger elastin sequences may offer advantages in terms of their kinetics of assembly or their mechanical properties. For example, MFU-2, which consists of exons 20, 21, 23, 24, 21, 23, and 24 of human elastin, has been expressed and purified. The amino acid sequence of this peptide is set forth in Figure 4C (SEQ ID NO:2). MFU-2 demonstrates an increased tendency towards spontaneous self-aggregation than MFU-1, as evidenced by a lower coacervation temperature. See Example 6 below. The amino acid sequence of MFU-5 (Fig. 5C; SEQ ID NO:11) corresponds to that of MFU-2 without the first amino acid residue.

15. At page 18, first full paragraph, please delete the entire paragraph and insert the following:

MFUs modeled on human elastin according to the present invention also can be used in any way that human or animal elastin is used. For example, the soluble MFUs of human elastin of the present invention can be used to coat the surfaces of non-biological materials, such as prothesis, in the same manner that solubilized (*i.e.*, hydrolyzed) non-human elastin preparations, such as animal alpha- and kappa-elastins, have been used. MFUs can be used to coat any

prosthesis, including a prosthesis comprising a synthetic material, an animal materials, and/or a metal. The prostheses can be coated with many layers of MFUs. For example, from 1 layer to 500 or more layers of MFU can be coated onto a prosthesis. The MFUs can be crosslinked after being coated onto the prosthesis to improve the permanence of the coating. As used herein, the term prosthesis is meant to encompass any material that is implanted into the body, including material for blood vessel replacement, for tissue replacement (i.e., "filler" material implanted after tissue loss), for heart valve replacement, cloth-like material, stents, and materials for use as coverings for burns or wounds to promote healing.

16. At page 18, second full paragraph, please delete the entire paragraph and insert the following:

Because the MFU's of the present invention are non-thrombogenic, and provide a surface on which endothelial and other cells can adhere and grow, prostheses coated with MFUs are more biocompatible than an uncoated prosthesis. Coating synthetic prosthesis with MFUs modeled on human elastin significantly inhibits platelet binding and activation. Moreover, prostheses coated with MFUs have the advantage over prosthesis coated with animal-derived elastin of containing a human sequence and, hence, being non-immunogenic. Also, the MFUs comprise a defined, homogeneous peptide rather than an undefined mixture of peptides of various sizes, like the animal-derived products previously described.

17. At page 22, second full paragraph, please delete the entire paragraph and replace with the following:

This particular unit was chosen because the flanking hydrophobic exon, exon 24, contains a seven-fold repeat of a PGVGVA (SEQ ID NO:6) sequence which is likely to play a role in elastin alignment and assembly. The importance of this domain is supported by the fact that domains of similar tandem repeats at this site are found in elastins of several species, and by the evidence that synthetic peptides mimicking this hydrophobic repeat sequence self-aggregate to form fibrillar structures. Also, the PGVGVA sequence (SEQ ID NO:6) interacts specifically with an elastin-binding protein, one of the functions of which is to prevent premature intracellular self-aggregation of tropoelastin. Hinek *et al.*, *J. Cell Biol.* 126: 563-73 (1994). This tropoelastin-binding protein has also been shown to inhibit *in vitro* self-aggregation of solubilized elastin fragments (kappa-elastin). Hinek, *Cell Adhesion & Comm.* 2: 1-9 (1994).

17. At page 22, line 36 – page 23, line 4, please delete the entire paragraph and replace with the following:

Peptides comprising other hydrophobic domains of human elastin are expected to posses similar abilities to self-assemble and self-align, and are suitable MFUs in accordance with the present invention. For example, peptides comprising amino acid residues 19-160, 188-367 and 607-717 of the human elastin amino acid sequence set forth in Figure 1B (SEQ ID NO:1) are suitable MFUs.

18. At page 28, first full paragraph, please delete the entire paragraph and replace with the following:

Example 7. Expression of MFUs Based on Lamprin and Elastin/Lamprin

Via techniques similar to those described in Example 2 above, constructs consisting of the entire polypeptide sequence of lamprin were expressed. A chimeric construct consisting of a crosslinking domain of human elastin (exons 21 and 23) flanked on both sides by tandem repeat sequences from lamprin, (GGLGY; SEQ ID NO:8)6, also was expressed.

IN THE CLAIMS

Please cancel claims 1-15 and 17-26 without prejudice or disclaimer, amend claim 16 and add the following new claims:

16. (Amended) A cosmetic material comprising a polypeptide that comprises an amino acid sequence consisting of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) that comprises at least three beta-sheet/beta-turn structures and at least one amino acid residue that participates in cross-linking, and that is not a naturally occurring fibrous protein.

27. (New) The cosmetic material of claim 16, wherein the polypeptide comprises an amino acid sequence selected from the group of amino acid sequences consisting of amino acid residues 374-499 of Figure 1B (SEQ ID NO:1), amino acid residues 19-160 of Figure 1B (SEQ ID NO:1), amino acid residues 188-367 of Figure 1B (SEQ ID NO:1), and amino acid residues 607-717 of Figure 1B (SEQ ID NO:1).

28. (New) The cosmetic material of claim 27, wherein the amino acid sequence of the polypeptide consists essentially of an amino acid sequence selected from the group

consisting of amino acid residues 374-499 of Figure 1B (SEQ ID NO:1), amino acid residues 19-160 of Figure 1B (SEQ ID NO:1), amino acid residues 188-367 of Figure 1B (SEQ ID NO:1), and amino acid residues 607-717 of Figure 1B (SEQ ID NO:1).

29. (New) The cosmetic material of claim 16, wherein the portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) is modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues.

30. (New) The cosmetic material of claim 16, wherein the polypeptide comprises tandem repeats of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1).

31. (New) The cosmetic material of 30, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in Figure 4C (SEQ ID NO:2), Figure 5A (SEQ ID NO:9), Figure 5B (SEQ ID NO:10) and Figure 5C (SEQ ID NO:11).

32. (New) The cosmetic material of claim 30, wherein the amino acid sequence of the polypeptide consists essentially of an amino acid sequence selected from the group consisting of the amino acid sequences set forth in Figure 4C (SEQ ID NO:2), Figure 5A (SEQ ID NO:9), Figure 5B (SEQ ID NO:10) and Figure 5C (SEQ ID NO:11).

33. (New) The cosmetic material of claim 30, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in Figure 4C (SEQ ID NO:2), Figure 5A (SEQ ID NO:9), Figure 5B (SEQ ID NO:10) and Figure 5C (SEQ ID NO:11), wherein the sequence is modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues.

IN THE DRAWINGS:

Figures 5A-5C are added to the application.

REMARKS

Amended claim 16 and new claims 27-33, directed to a cosmetic material, are pending in the present application. The specification is amended to include priority claims and additional embodiments of the invention which were previously described in the parent application, U.S. serial application No. 09/340,736. Claims 1-15 and 17-26 are canceled without prejudice or disclaimer as being drawn to subject matter allowed in U.S. patent No. 5,969,106. Original claims 27-46 were added and presented for examination by Preliminary Amendment in the parent application, U.S. serial application No. 09/340,736. Due to a restriction requirement, claims 16 and 27-33 were cancelled as non-elected matter and claims 34-36 are pending in the parent application.

Accordingly, Applicants cancel claims 34-46 without prejudice and disclaimer, and present amended claims 16 and new claim 27-33 for the examination on the merits. An early notice in this regard is earnestly solicited. Should there be any questions regarding this application, the Examiner is invited to contact the undersigned at the telephone number shown below.

Respectfully submitted,

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Reg No 34,717

for

Stephen A. Bent
Reg. No. 29,768

September 28, 2001
Date

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Should additional fees be necessary in connection with the filing of this divisional application and Preliminary Amendment, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees.

MARKED UP VERSION SHOWING CHANGES MADE

IN THE SPECIFICATION

1. At page 1, after the Title, please insert the following information:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application serial No. 09/340,736, filed June 29, 1999, which is a continuation-in-part of U.S. patent application serial No. 08/911,364, filed August 7, 1997, now U.S. patent No. 5,969,106, which, in turn, is based on U.S. provisional patent application serial No. 60/023,522, filed August 7, 1996. The entire contents of these applications, including their specifications, claims and drawings, are incorporated herein by reference in their entirety.

2. At page 2, line 36 – page 3, line 10, please delete the entire paragraph and replace with the following:

U.S. Patent No. 4,474,851 (Urry) is directed to an elastomeric composite material comprising an artificial core fiber, such as Dacron, and a polypeptide comprising repeating tetrapeptide or pentapeptide units. The units are derived from units observed to be repeated in the tropoelastin molecule, Val-Pro-Gly-Val-Gly (VPGVG; SEQ ID NO:6) and Val-Pro-Gly-Gly (VPGG; SEQ ID NO:7). The polypeptide comprises a series of beta-turns and is proposed to have a beta-coil structure. The polypeptide provides elastomeric properties to the composite material, but has little structural strength or integrity. The artificial core fiber provides these latter properties to the composite material.

3. At page 3, second full paragraph, please delete the entire paragraph and replace with the following:

Elastin-based materials also have been used to produce solid materials from which prostheses can be manufactured. These include soluble animal elastin co-aggregated with other proteins such as collagen, fibrin, fibronectin and laminin, to produce gel-like materials, and polymerized materials derived from short hydrophobic sequences of human elastin (such as PGVGVA; SEQ ID NO:5). In some cases, these synthetic peptides also include short alanine-rich sequences containing lysine residues, allowing cross-linking between the elastin-like peptides or to other proteins such as collagen. Both elastin and collagen contain crosslinks derived from lysine. For example, U.S. Patent No. 5,223,420 (Rabaud) is directed

to an elastin-based product comprising an adduct containing elastin and at least one other protein, such as fibrin.

4. At page 3, line 32 – page 4, line 7, please delete the entire paragraph and replace with the following:

U.S. Patent No. 4,589,882 (Urry) is directed to an artificial elastomeric copolymer comprising an elastomeric component of repeating units of tetrapeptides and pentapeptides and a crosslinking component which may comprise amino acid residues. The repeating units are derived from elastin. U.S. Patent No. 4,132,746 (Urry) is directed to a synthetic, insoluble, crosslinked polypentapeptide. The pentapeptide is the VPGVG (SEQ ID NO:6) peptide present in tropoelastin. See also U.S. Patent No. 4,500,700, U.S. Patent No. 4,870,055, and U.S. Patent No. 5,250,516 (all to Urry) for other materials derived from this peptide. The polypeptides described in these patents comprise a series of beta-turns and are proposed to have a beta-coil structure.

5. At page 6, lines 34-37, please delete the entire paragraph and replace with the following:

Figure 1B shows the amino acid sequence of human elastin (SEQ ID NO:1), without the signal peptide. The underlined amino acid residues comprise the polypeptide of the present invention named MFU-1.

6. At page 7, lines 15-17, please delete the entire paragraph and replace with the following:

Figure 4C shows the amino acid sequence of MFU-2 (SEQ ID NO:2).

7. At page 7, line 21, please insert the following paragraph:

Figures 5A, 5B and 5C show the amino acid sequences of MFU-3 (SEQ ID NO:9), MFU-4 (SEQ ID NO:10), and MFU-5 (SEQ ID NO:11), respectively.

8. At page 8, line 32 – page 9, line 5, please delete the entire paragraph and replace with the following:

Tropoelastin consists predominantly of alternating hydrophobic and crosslinking domains. Indik *et al.*, *Proc. Nat'l Acad. Sci. USA* 84: 5680-84 (1986). Crosslinking domains

are rich in alanine (A), with the lysines (K) destined for involvement in crosslink formation present in KAAK (SEQ ID NO:3) and KAAAK (SEQ ID NO:4) spacings. The domains separating these crosslinking regions are strongly hydrophobic in character, and contain many tandemly repeated penta- and hexa-peptide sequences. In human elastin the most striking of these is the sequence PGVGVA (SEQ ID NO:6), repeated 7 times in exon 24. Indik *et al.*, *supra*.

9. At page 9, line 31 – page 10, line 17, please delete the entire paragraph and replace with the following:

As shown in Figure 1A, human elastin consists for most of its length of alternating crosslinking domains and hydrophobic domains. The crosslinking domains consist mainly of lysine (K) and alanine (A) residues in KAAK (SEQ ID NO:3) and KAAAK (SEQ ID NO:4) sequences, wherein the lysine residues are in a suitable conformation for oxidative deamination by lysyl oxidase and subsequent formation of the covalent desmosine crosslinks. Indik *et al.*, *supra*. The hydrophobic domains are rich in hydrophobic pentapeptide and hexapeptide sequences believed to be in beta-sheet/beta-turn structures. Tamburro *et al.*, ADVANCES IN LIFE SCIENCES 115-27 (1990). These hydrophobic regions are believed to be important to elastin's physical properties of extensibility and elastic recoil, and to the ability of tropoelastin (the monomeric precursor of elastin) to self-aggregate into fibrillar structures. Robson *et al.*, *supra*; Tamburro *et al.*, *supra*. Other proteins capable of self-aggregation and self-alignment into stable fibrillar matrices, including eggshell chorion proteins of insects, spider dragline silk, and lamprin from lamprey cartilage, all possess similar regions of hydrophobic repeat peptides with beta-sheet/beta-turn structures. Hamodrakas *et al.*, *Int. J. Biol. Macromol.* 11: 307-13 (1989); Simmons *et al.*, *Science* 271: 84-87 (1996); Robson *et al.*, *supra*.

10. At page 13, line 23 to page 14, line 16, please delete the entire paragraph and replace with the following:

In accordance with one embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1). The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1), wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10

amino acid residues, for example, from 1 to about 5 amino acid residues . Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. In accordance with another embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of the amino acid sequence set forth in Figure 4C (SEQ ID NO:2). The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid sequence set forth in Figure 4C (SEQ ID NO:2), wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10 amino acid residues, for example, from 1 to about 5 amino acid residues. Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. Polypeptides whose amino acid sequences are variants of the amino acid sequences set forth in Figures 5A-5C (SEQ ID NOS:9-11, respectively) also are encompassed by the present invention. The amino acid sequences of such polypeptides comprise a portion of an amino acid sequence set forth in Figure 5A, 5B or 5C (SEQ ID NOS:9, 10 or 11, respectively), wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues, for example, from 1 to about 5 amino acid residues. Such polypeptides have a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibit the properties of self-alignment discussed herein.

PCT/US02/05512

11. At page 14, second full paragraph, please delete the entire paragraph and replace with the following:

The domain structure of human elastin is illustrated in Figure 1A. As shown in this Figure, there are a number of alternating crosslinking and hydrophobic domains. The hydrophobic domains each are believed to comprise a number of beta-sheet/beta-turn-forming sequences. These domains represent probable MFUs of elastin. One of these, used in further experimentation, is designated by the bracket and is named MFU-1 (see Example 1 below). Figure 1B sets forth the amino acid (SEQ ID NO:1) of human elastin. The underlined amino acid residues, residues 374-499, comprise MFU-1. Other MFUs modeled on human elastin include polypeptides comprising amino acid residues 19-160, 188-367 and 607-717, respectively. The amino acid sequence of MFU-3 (SEQ ID NO:9; Fig. 5A) corresponds to that of MFU-1 without the first five amino acid residues. The amino acid sequence of MFU-4 (SEQ ID NO:10; Fig. 5B) corresponds to that of MFU-1 without the first four amino acid residues.

12. At page 14, line 33 – page 15, line 5, please delete the entire paragraph, and replace with the following:

MFUs modeled on human elastin comprise a portion of the amino acid sequence of the tropoelastin molecule (Figure 1B; SEQ ID NO:1) and have at least three beta-sheet/beta-turn structures in their secondary structure. They also may comprise amino acids residues which are capable of participating in crosslinking, such as lysine residues. In one embodiment of the invention, the MFU comprises two amino acid residues capable of participating in crosslinking in such a manner as to form a desmosine-type linkage. For example, the MFU may comprise a KAAK (SEQ ID NO:3) or KAAAK (SEQ ID NO:4) amino acid sequence.

13. At page 15, first full paragraph, please delete the entire paragraph and replace with the following:

In a preferred embodiment, a polypeptide modeled on human elastin consists essentially of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1). The phrase "A consists essentially of B" herein denotes that A comprises B and possibly other components that do not materially affect the characteristics of the A-B material. For example, a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) denotes a polypeptide which comprises a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) and which also may comprise other amino acid residues that do not materially alter the characteristics of the polypeptide. That is, the polypeptide maintains the characteristics of having at least three beta-sheet/beta-turn structures, and self-aligning in the same manner as tropoelastin peptides.

14. At page 16, first full paragraph, please delete the entire paragraph and replace with the following:

By means of available technology, DNA sequences coding for tandem repeats of any human elastin MFU, or for MFUs containing larger domains of human elastin, up to and including the entire tropoelastin molecule, can be constructed. These larger elastin sequences may offer advantages in terms of their kinetics of assembly or their mechanical properties. For example, MFU-2, which consists of exons 20, 21, 23, 24, 21, 23, and 24 of human elastin, has been expressed and purified. The amino acid sequence of this peptide is set forth in Figure 4C (SEQ ID NO:2). MFU-2 demonstrates an increased tendency towards

spontaneous self-aggregation than MFU-1, as evidenced by a lower coacervation temperature. See Example 6 below. The amino acid sequence of MFU-5 (Fig. 5C; SEQ ID NO:11) corresponds to that of MFU-2 without the first amino acid residue.

15. At page 18, first full paragraph, please delete the entire paragraph and insert the following:

MFUs modeled on human elastin according to the present invention also can be used in any way that human or animal elastin is used. For example, the soluble MFUs of human elastin of the present invention can be used to coat the surfaces of non-biological materials, such as prothesis, in the same manner that solubilized (*i.e.*, hydrolyzed) non-human elastin preparations, such as animal alpha- and kappa-elastins, have been used. MFUs can be used to coat any prosthesis, including a prosthesis comprising a synthetic material, an animal materials, and/or a metal. The prostheses can be coated with many layers of MFUs. For example, from 1 layer to 500 or more layers of MFU can be coated onto a prosthesis. The MFUs can be crosslinked after being coated onto the prosthesis to improve the permanence of the coating. As used herein, the term prosthesis is meant to encompass any material that is implanted into the body, including material for blood vessel replacement, for tissue replacement (i.e., "filler" material implanted after tissue loss), for heart valve replacement, cloth-like material, stents, and materials for use as coverings for burns or wounds to promote healing.

16. At page 18, second full paragraph, please delete the entire paragraph and insert the following:

Because the MFU's of the present invention are non-thrombogenic, and provide a surface on which endothelial and other cells can adhere and grow, prostheses coated with MFUs are more biocompatible than an uncoated prosthesis. Coating synthetic prosthesis with MFUs modeled on human elastin significantly inhibits platelet binding and activation. Moreover, prostheses coated with MFUs have the advantage over prosthesis coated with animal-derived elastin of containing a human sequence and, hence, being non-immunogenic. Also, the MFUs comprise a defined, homogeneous peptide rather than an undefined mixture of peptides of various sizes, like the animal-derived products previously described.

17. At page 22, second full paragraph, please delete the entire paragraph and replace with the following:

This particular unit was chosen because the flanking hydrophobic exon, exon 24, contains a seven-fold repeat of a PGVGVA (SEQ ID NO:6) sequence which is likely to play a role in elastin alignment and assembly. The importance of this domain is supported by the fact that domains of similar tandem repeats at this site are found in elastins of several species, and by the evidence that synthetic peptides mimicking this hydrophobic repeat sequence self-aggregate to form fibrillar structures. Also, the PGVGVA sequence (SEQ ID NO:6) interacts specifically with an elastin-binding protein, one of the functions of which is to prevent premature intracellular self-aggregation of tropoelastin. Hinek *et al.*, *J. Cell Biol.* 126: 563-73 (1994). This tropoelastin-binding protein has also been shown to inhibit *in vitro* self-aggregation of solubilized elastin fragments (kappa-elastin). Hinek, *Cell Adhesion & Comm.* 2: 1-9 (1994).

17. At page 22, line 36 – page 23, line 4, please delete the entire paragraph and replace with the following:

Peptides comprising other hydrophobic domains of human elastin are expected to posses similar abilities to self-assemble and self-align, and are suitable MFUs in accordance with the present invention. For example, peptides comprising amino acid residues 19-160, 188-367 and 607-717 of the human elastin amino acid sequence set forth in Figure 1B (SEQ ID NO:1) are suitable MFUs.

18. At page 28, first full paragraph, please delete the entire paragraph and replace with the following:

Example 7. Expression of MFUs Based on Lamprin and Elastin/Lamprin

Via techniques similar to those described in Example 2 above, constructs consisting of the entire polypeptide sequence of lamprin were expressed. A chimeric construct consisting of a crosslinking domain of human elastin (exons 21 and 23) flanked on both sides by tandem repeat sequences from lamprin, (GGLGY; SEQ ID NO:8)6, also was expressed.

IN THE CLAIMS

Please cancel 1-15, and 17-26 without prejudice or disclaimer, and amend claim 16 as follows:

16. (Amended) A cosmetic material comprising **a** ~~**[the]**~~ polypeptide ~~**[of claim 5]**~~ **that comprises an amino acid sequence consisting of a portion of the amino acid sequence set forth in Figure 1B (SEQ ID NO:1) that comprises at least three beta-sheet/beta-turn structures and at least one amino acid residue that participates in cross-linking, and that is not a naturally occurring fibrous protein**.

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Atty. Dkt. No. 041082-0113

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Aser ROTHSTEIN et al.

Title: SELF-ALIGNING PEPTIDES
MODELED ON HUMAN ELASTIN
AND OTHER FIBROUS PROTEINS

Appl. No.: Unassigned

Filing Date: 09/28/2001

Examiner: Unassigned

Art Unit: Unassigned

09956512 - 092801

TRANSMITTAL OF FORMAL DRAWINGS

Commissioner for Patents
Washington, D.C. 20231

ATTENTION: DRAWING REVIEW BRANCH

Sir:

Transmitted herewith are the formal drawings (6 sheets, Figures 1-5C) for the above-identified application. The Official Draftsperson is respectfully requested to approve these drawings for entry into the application.

Respectfully submitted,

Date: September 28, 2001

By _____

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09/964662
09/28/01

FIGURE 1A

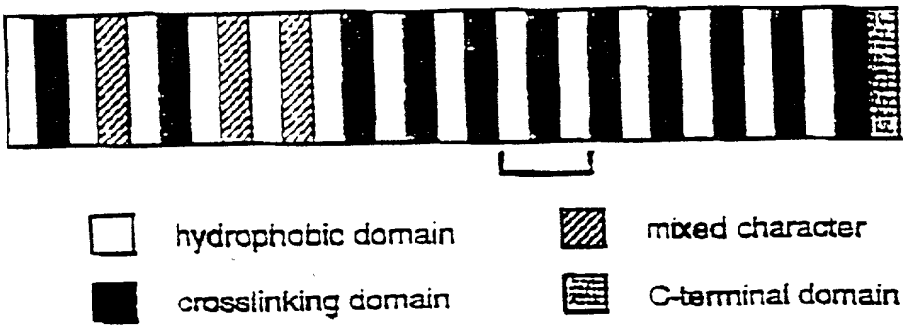

FIGURE 1B

```
1           11          21          31          41          51
GGVPGAIPGG VPGGVPYPGA GLGALGGGAL GPGGKPLKPV PGGLAGAGLG AGLGAFPAVT
FPGALVPGGV ADAAAAAYKA KAGAGLGGVP GVGGLGVSAG AVVPQPGAGV KPGKVPGVGL
PGVYPGGVLP GARFPGVGVL PGVPTGAGVK PKAPGVGGAF AGIPGVGPFG GPQPGVPLGY
PIKAPKLPGG YGLPYTTGKL PYGYGPGGVA GAAGKAGYPT GTGVGPQAAA AAAAKAAAKF
GAGAAGVLPG VGGAGVPGVP GAIPGIGGIA GVGIPAAAAA AAAAAKAAKY GAAAGLVPGG
PGFGPGVVGV PGAGVPGVGV PGAGIPVVPG AGIPGAAVPG VVSPEAAAKA AAKAAKYGAR
PGVGVGGIPT YGVGAGGFPG FGVGVGGIPG VAGVPSVGGV PGVGGVPGVG ISPEAQAAAA
AKAAKYGVGT PAAAAAAKAA KAAQFGLVPG VGVAPGVGVA PGVGVAPGVG LAPGVGVAPG
VGVAPGVGVA PGIGPGGVAA AAKSAAKVAA KAQLRAAAGL GAGIPGLGVG VGVPGLGVGA
GVPGLGVGAG VPGFGAGADE GVRRSLSPEL REGDPSSSQH LPSTPSSPRV PGALAAAKAA
KYGAAVPGVL GGLGALGGVG IPGGVVGAG PAAAAAAAKAA AKAAQFGLVG AAGLGGLGVG
GLGVPGVGGL GGIPPAAAAK AAKYGAAGL GGVLGGAGQFP LGGVAAPPGF GLSPIFPGGA
CLGKACGRKR K
```

FIGURE 1C

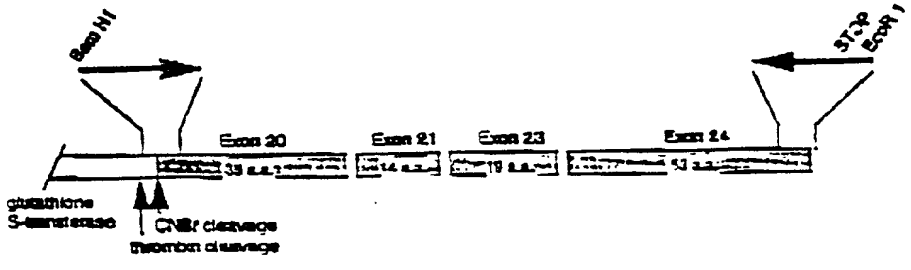

FIGURE 1D

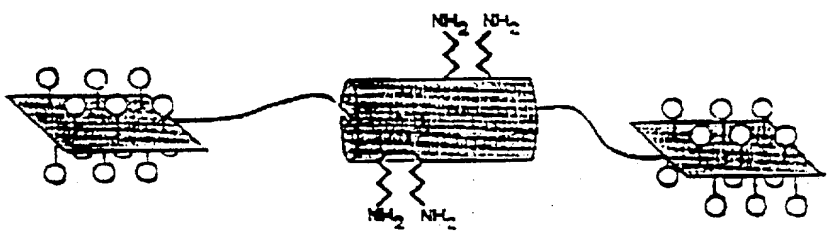

FIGURE 1E

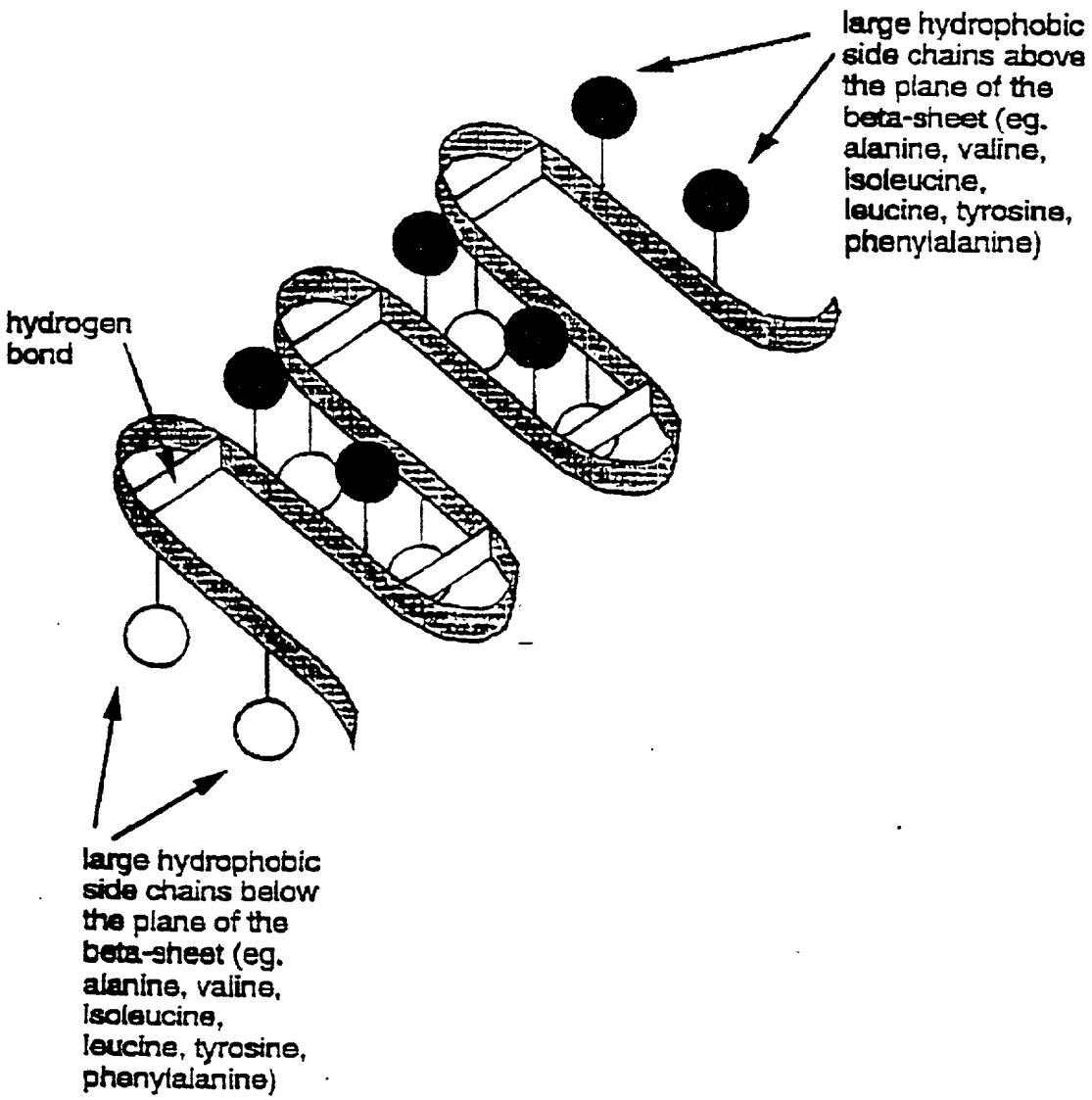
large hydrophobic side chains above the plane of the beta-sheet (eg. alanine, valine, isoleucine, leucine, tyrosine, phenylalanine)
hydrogen bond
large hydrophobic side chains below the plane of the beta-sheet (eg. alanine, valine, isoleucine, leucine, tyrosine, phenylalanine)

FIGURE 2

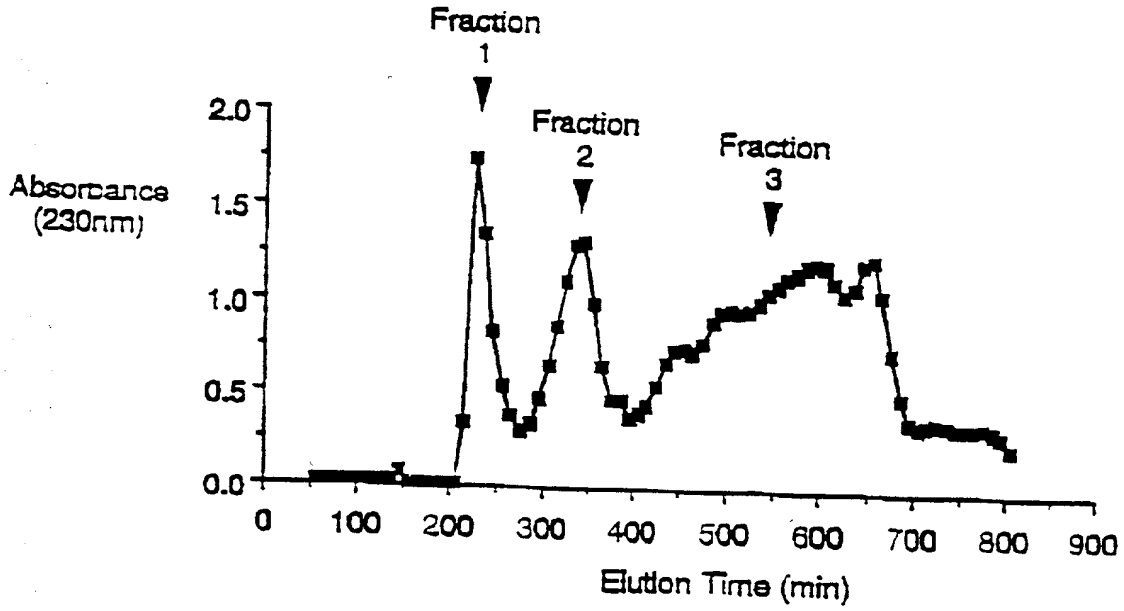
Fraction 1
Fraction 2
Fraction 3
Absorbance (230nm)
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
700
800
900
Elution Time (min)

Figure 3

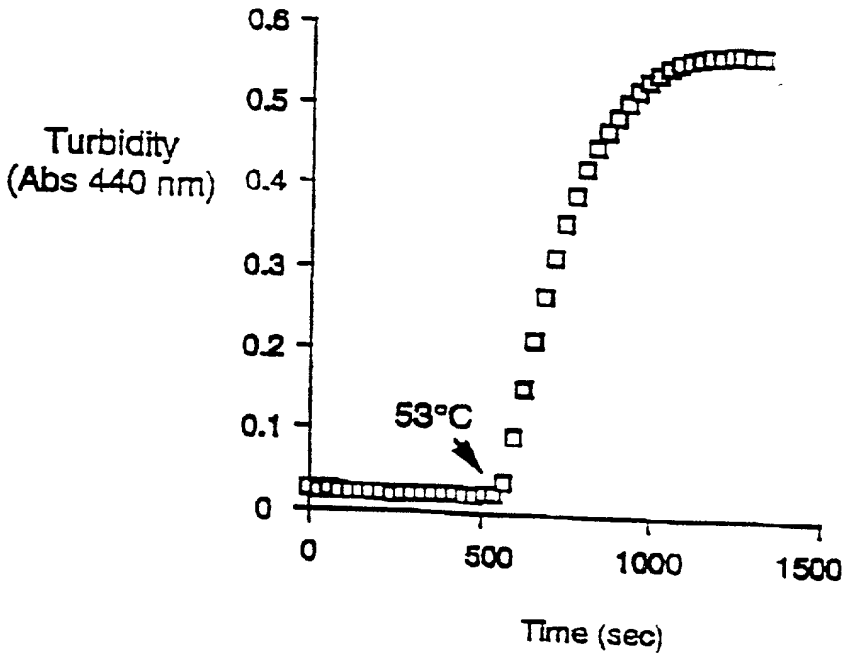
Turbidity
(Abs 440 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
53°C
0
500
1000
1500
Time (sec)

Figure 4A

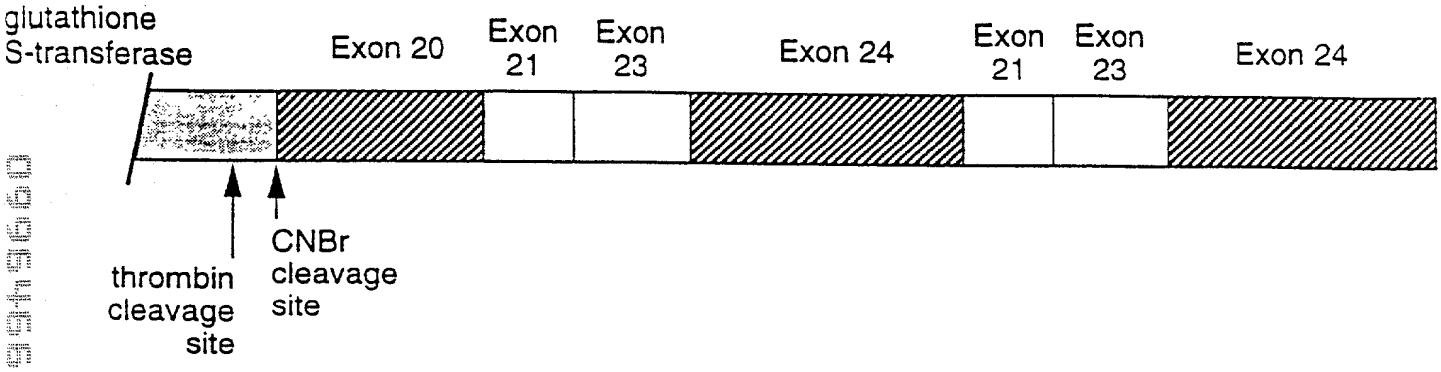

Figure 4B

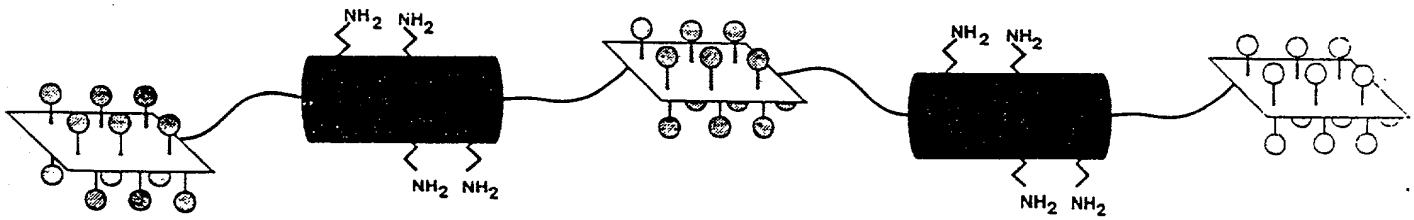

Figure 4C

FPGFGVGVGG IPGVAGVPGV GGVPGVGGVP GVGISPEAQA AAAAKAAKYG
VGTPAAAAAK AAAKAAQFGL VPGVGVAPGV GVAPGVGVAP GVGLAPGVGV
APGVGVAPGV GVAPAIGPPE AQAAAAAKAA KYGVGTPAAA AAKAAAKAAQ
FGLVPGVGVA PGVGVAPGVG VAPGVGLAPG VGVAPGVGVA PGVGVAPAIG P